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First insights into the molecular population structure and origins of the invasive Chinese sleeper, *Perccottus glenii*, in Europe

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Abstract

The aim of our study was to provide a first overview of the population genetic structure of the invasive Chinese sleeper, *Perccottus glenii*, (Actinopterygii: Odontobutidae) in European water bodies. This species originates from inland waters of north-eastern China, northern North Korea and the Russian Far East. The 1172 bp long portion of the cytochrome b gene was sequenced from Chinese sleeper specimens collected from a variety of water bodies in Belarus, Bulgaria, Hungary, Germany, Latvia, Lithuania, Poland, Russia (European part) and Ukraine. Our study revealed that the invasive Chinese sleeper in Europe consists of at least three distinct haplogroups that may represent independent introduction events from different parts of its native area; i.e. three founding populations: (1) Baltic haplogroup that may originate either from fish introduced inadvertent from Russia or from some unidentified source (release by aquarists). So far, this haplogroup has been found only in the Daugava basin in Latvia. (2) East-European haplogroup that may

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originate from an unintentional introduction to the Volga basin in Russia and has expanded westward. So far, this group was recorded in the Volga, Upper Dnieper and Neman drainages in Belarus, Lithuania, and Russia. (3) Carpathian haplogroup, that originated from individuals unintentionally introduced with Asian cyprinid fishes to Lviv region in Ukraine and are now widely distributed in Central Europe.

Keywords
Amur sleeper, exotic fish, invasion pathways, phylogeography

Introduction

Inland fisheries and fish farming are commercially important activities in many countries, but the associated risk management (such as quarantine control) is usually less rigid than is the case with other taxa (Copp et al. 2005). With increasing globalisation, the number of fish introductions has increased in recent decades, posing ecological and evolutionary threats to biodiversity (Hulme 2009; Vitule et al. 2009; Gozlan et al. 2010; Cucherousset and Olden 2011). The negative influence of alien species on endemic fauna includes predation, food and spatial competition, hybridization, the spread of parasites and pathogens, and modification of food chains (Leunda 2010; Cucherousset and Olden 2011).

The Chinese sleeper, *Perccottus glenii* Dybowski, 1877, formerly known as the Amur sleeper, is a successful alien freshwater fish species in European waters, with a high invasive potential (Negring and Steinhof 2015; European Commission 2016). Since its introduction at the beginning of the 20th century, the species has continuously expanded its invasive range in Eurasia (Reshetnikov 2010, 2013; Reshetnikov and Ficetola 2011). The native range of the Chinese sleeper encompasses inland waters of north-eastern China, northern North Korea and the Russian Far East, including the middle and lower stretches of the River Amur with the tributaries Zeya, Sungari, Ussuri as well as the Lake Khanka basin and drainages of the rivers Gou, Liaohe, Never, and Yalu (China, North Korea) (Mori 1936; Bogutskaya and Naseka 2002; Miller and Vasil'eva 2003; Kottelat and Freyhof 2007; Bogutskaya et al. 2008; Reshetnikov 2010). Some introduction events appear noteworthy in the history of the Chinese sleeper invasion in Europe. Specimens were first transported to Saint Petersburg from the River Zeya (Russian Far East) in 1912 (Kuderskiy 1982) by a scientific expedition as ornamental fish and subsequently kept in an aquarium (Nabatov 1914). In 1916 four individuals were released into a garden pond (Fig. 1A), where they founded a population that was reported to eradicate the entire local fish fauna (Dmitriev 1971). During the 1920s, the Chinese sleeper invaded many water bodies around Saint Petersburg (Kuderskiy 1982) and in the 1950s it was recorded in the shallow waters of the Gulf of Finland in the Baltic Sea (Dmitriev 1971).

Another introduction took place in 1950, when ichthyologists from Moscow State University and the Polar Institute of Marine Fisheries and Oceanography (PINRO) transported fish from the Amur River and released them into the Tarakanov and Ostankino ponds in Moscow (Reshetnikov 2004; Reshetnikov and Ficetola 2011) (Fig.

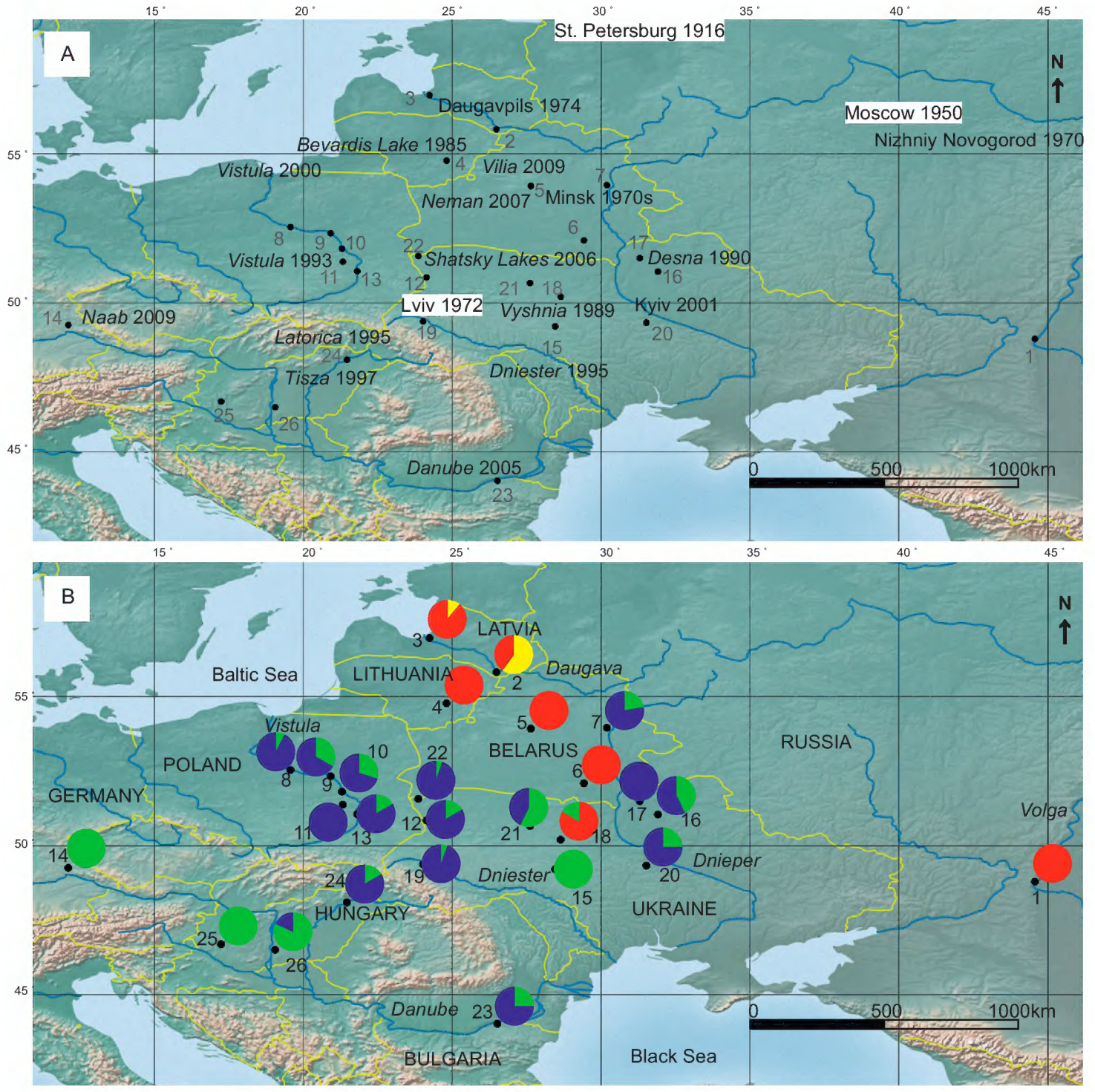

Figure 1. A history of the expansion of the Chinese sleeper in Europe (the earliest introduction is indicated and highlighted in white) in relation to the location of sampling sites (sites in close geographic proximity pooled for demographic analyses and assigned the same number) **B** distribution and proportional abundance of Chinese sleeper cytochrome b haplogroups in the study area. Haplogroup I (yellow), haplogroup II (red), haplogroup III (subgroup IIIa – green; subgroup IIIb – blue).

1A). A further invasion of Chinese sleeper is associated with the increasing popularity of Asian cyprinid cultivation in the former Soviet Union from the 1950s to the 1970s. During that time, Chinese sleepers were reported several times from fish ponds after inadvertent introduction with commercial cyprinids. The introductions have facilitated further invasion by active and passive dispersal (Reshetnikov 2010; Reshetnikov and Ficetola 2011).

The history of the Central European population of Chinese sleeper started in 1972, when it was found in the Velykyi Lubin fish farm (River Dniester basin) near Lviv, Ukraine (Fedoniuk 2005; Reshetnikov 2013; Kutsokon 2017) (Fig. 1A). Ac-

cording to Reshetnikov (2013), this was the most probable secondary source of the invasion to several other countries in Central Europe, given that it was subsequently reported from Poland in 1993 (Antychowicz 1994), Hungary in 1997 (Harka 1998), Slovakia in 1998 (Koščo et al. 1999), Bulgaria in 2003 (Jurajda et al. 2006), Romania in 2005 (Popa et al. 2006), and Germany in 2009 (Reshetnikov and Schliewen 2013; Nehring and Steinhof 2015).

In the invaded areas, Chinese sleepers are locally abundant, especially in small, stagnant and eutrophic water bodies that are overgrown with vegetation, such as oxbow lakes, floodplain pools, bogs and ponds, both natural and artificial (Koščo et al. 2003; Grabowska et al. 2011; Reshetnikov 2013; Rechulicz et al. 2015). They appear to avoid running waters, though they may temporally occur in flowing water or occupy habitats that provide shelter from the current. Their invasion of new localities still continues, via both active and passive dispersal (Reshetnikov 2013). Human activity, such as fish cultivation, is considered one of the primary reasons for accidental introduction of the Chinese sleeper to geographically distant locations (Reshetnikov 2013). Given that the species is still treated as both an ornamental and baitfish, another suspected human-mediated explanation for its dispersal is a release by aquarists and anglers (Reshetnikov 2013; Rakauskas et al. 2016b). Anthropogenic introductions have facilitated further expansion via natural mechanisms, particularly through drainage ditches, streams and rivers that seem to serve as invasion highways at river drainage scale (Reshetnikov 2013). The Chinese sleeper is capable of depleting the diversity of macroinvertebrates, amphibians, reptiles, and fishes (Koščo et al. 2008; Grabowska et al. 2009, 2019; Pupins and Pupina 2012, 2018; Reshetnikov, 2013; Rakauskas et al. 2016a), making it a serious threat to European freshwater ecosystems. In consequence, it was included in the invasive alien species list of European Union concern (European Commission 2016).

The genetic diversity and population structure of the Chinese sleeper are poorly known, though data were recently collected for part of its native range in China in the River Amur and Liaohe basins (Xu et al. 2013, 2014). These data showed the presence of three Chinese sleeper lineages that supposedly diverged during the Pleistocene; two of them were sympatric in both basins, while the third was restricted to the Amur basin. In its native range, the Chinese sleeper was characterised by a high degree of genetic structure among populations, which can be attributed to its limited dispersal (Xu et al. 2014). So far, with the exception of a single ambiguous study on a population from the River Siret in Romania (Luca et al. 2014), there have been no attempts to reveal the genetic structure and possible origins of the Chinese sleeper populations in its invasive range.

Our study is the first to address this gap in understanding about such a widespread and important invasive species, and is based on samples collected from almost all European countries that currently support populations of the Chinese sleeper. Our aim is to provide a better understanding of the dynamics, pathways, and vectors of the expansion of the Chinese sleeper in Europe. In particular we aimed to: 1) test whether the invasive population of the Chinese Sleeper in Central Europe comes from one or

several introduction events and their sources; 2) detect, on the basis of already published data, the source of European populations in its native range; 3) verify whether, as suggested by the literature, Ukraine is the location of the initial introduction and a donor for subsequent expansion into Central Europe; 4) discuss the pathways and vectors that could play a role in driving the expansion of the Chinese sleeper in Europe and shaping its genetic structure.

Materials and methods

DNA extraction, amplification, sequencing, and dataset assemblage

Total DNA was extracted from 261 individuals collected on 26 sampling sites in Central (Germany, Poland, Hungary) and Eastern Europe (Latvia, Lithuania, Belarus, Ukraine, Russia and Bulgaria) (Fig. 1A, B, Table 1), from a piece of a fin tissue, with the Chelex (Casquet et al. 2012) or standard phenol-chloroform (Hillis et al. 1996) procedures. The ca. 1200 bp long fragment of cytochrome b (cyt b) gene was amplified using the primer pair Glu-2 and Pro-R1 and reaction conditions of Hardman and Page (2003). The PCR products (5 µl) were cleaned with Exonuclease I (2 U, EURx Ltd., Gdańsk, Poland) and alkaline phosphatase Fast Polar-BAP (1 U, EURx Ltd., Gdańsk, Poland) treatment, according to the manufacturer's guidelines, and sent for sequencing in both directions to Macrogen Europe (Amsterdam, the Netherlands). The identity of the obtained sequences was verified using BLAST (Altschul et al. 1990). Sequences were edited, aligned and trimmed to 1172 bp using Geneious 10.2.6 (Kearse et al. 2012). In the case of six individuals, the chromatograms contained double peaks. These double peaks were confirmed by sequencing both DNA strands (5' to 3' and 3' to 5'). Un-phasing of such sites in order to define the actual haplotypes was completed in DnaSP v5 (Librado and Rozas 2009). The sequences of the resulting haplotypes were translated into amino acid sequences using Geneious 10.2.6 to check for the presence of stop codons that could identify them as NUMT-pseudogenes. No such cases were found and we assumed that they represented fully functional versions of the mitochondrial COI genes. All the sequences were deposited in GenBank under accession numbers (MN555819–MN556085). The DNA sequences containing relevant voucher information were deposited in the online database of the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007) accessible through the public data set "PEGLE" (dx.doi.org/10.5883/DS-PERCCOTT). All specimens were deposited in the permanent collection of the Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Poland. Having no possibility to validate the tentative heteroplasmy by repeating DNA extractions from the samples in question, we have excluded the sequences MN555836, MN555846, MN555858, MN555862, MN555864, MN555959, MN556020, MN556038, MN556052, MN556058, MN556059, MN556064 from subsequent analyses. Hence, the final dataset was composed of 255 cytochrome b sequences.

Table 1. Sampling sites of Chinese sleeper. Localities geographically very close (ca. 20 km distance) to each other were pooled to simplify the demographic analyses. They were given the same site number.

Site	Code	Country	Latitude / Longitude	Date	Drainage	Locality	No. of individuals	No. of sequences
1	RUS1	Russia	48.7953, 44.5641	2018	Volga	Volgograd	10	10
2	LAT1	Latvia	55.8348, 26.4843	2017	Daugava	Daugavpils city	10	10
3	LAT2	Latvia	56.9772, 24.2409	2017	Daugava	Riga city	9	9
4A	LT1	Lithuania	54.7835, 24.8125	2019	Nemunas	Neris River drainage	9	9
4B	LT2	Lithuania	54.8430, 25.3406	2019	Nemunas	Neris River drainage	4	4
5	BLR1	Belarus	53.9329, 27.6398	2017	Dnieper	Minsk	10	10
6	BLR2	Belarus	52.0994, 29.4222	2017	Dnieper	Syrod – Gomel oblast	12	12
7	BLR3	Belarus	53.9621, 30.1924	2017	Dnieper	Mogilev oblast	9	9
8	PL1	Poland	52.5459, 19.5659	2018	Vistula	Wloclawski Reservoir	13	13
9	PL2	Poland	52.3356, 20.9142	2018	Vistula	Łomianki	12	12
10	PL3	Poland	51.8206, 21.2942	2018	Vistula	Pilica River, Zagroby	11	12
11	PL4	Poland	51.3843, 21.3227	2018	Vistula	Niemianowice	11	11
12	PL5	Poland	50.8569, 24.1415	2018	Vistula	Western Bug Zosin	3	3
12	PL6	Poland	50.5376, 23.7342	2018	Vistula	Western Bug Nadolce	3	3
13	PL7	Poland	51.0663, 21.8140	2018	Vistula	Łopoczno	10	10
14	GER1	Germany	49.2630, 12.1096	2015	Danube	Kranzloh fish pond	7	7
14	GER2	Germany	49.2647, 12.1298	2015	Danube	Torngrube	7	7
15	UA1	Ukraine	49.2158, 28.4578	2016	Southern Bug	Vinnytsia	1	1
15	UA3	Ukraine	49.2158, 28.4578	2018	Southern Bug	Vinnytsia region	3	3
16	UA2	Ukraine	51.0528, 31.9069	2018	Dnieper	Desna River Nizhyn	7	7
17	UA4	Ukraine	51.5041, 31.2943	2016	Dnieper	Desna River Chernihiv	2	2
18	UA5	Ukraine	50.2070, 28.6420	2016	Dnieper	Teteriv River, Huiva	1	1
18	UA11	Ukraine	50.5103, 29.3078	2018	Dnieper	Bereztsi	6	7
19	UA6	Ukraine	49.3827, 24.0204	2017	Dniester	River Kuna basin, Lviv oblast	3	3
19	UA7	Ukraine	49.8483, 24.0521	2017	Dniester	Lviv oblast	4	4
19	UA18	Ukraine	49.8001, 24.0164	2018	Dniester	Stryiska Pond, Lviv	11	11
20	UA8	Ukraine	49.3395, 31.5156	2016	Dnieper	Dnipro, Mliiv	1	1
20	UA9	Ukraine	50.3511, 30.4557	2018	Dnieper	Novosilky – pond	3	3
21	UA10	Ukraine	50.6673, 27.6121	2018	Dnieper	Chyzhivka	11	15
22	UA12	Ukraine	51.5774, 23.8628	2018	Vistula/Dnieper	Shatsk Lakes, Canal in Zatyshshia	6	6
22	UA13	Ukraine	51.5543, 23.9179	2018	Vistula/Dnieper	Shatsk Lakes, Canal in Melnyky	4	4
22	UA14	Ukraine	51.5488, 23.9221	2018	Vistula/Dnieper	Shatsk Lakes, Melnyky Pond	1	1
22	UA15	Ukraine	51.5270, 23.8527	2018	Vistula/Dnieper	Shatsk Lakes, Canal in Illichivka,	2	2
22	UA17	Ukraine	51.5774, 23.8628	2018	Vistula/Dnieper	Shatsk Lakes, Canal in Zatyshshia	5	5
23	BUL1	Bulgaria	44.0267, 26.5170	2015	Danube	Kalimok	8	8
24	H1	Hungary	48.0953, 21.4629	2018	Danube	Rakamaz oxbow lake, Tiszanagyfalu	12	12
25	H2	Hungary	46.6939, 17.2373	2018	Danube	Canal in Kis-Balaton reservoir, Fenékpuszta	9	9
26	H3	Hungary	46.5063, 19.0531	2018	Danube	Maloméri main canal, Homokmégy	11	11

Analysis of molecular data

Haplotypes within the cytochrome b dataset as well diversity statistics; i.e. the number of haplotypes (k), haplotypic diversity (h) and nucleotide diversity (Pi) (Nei 1987), were obtained using the DnaSP v5 software (Librado and Rozas 2009).

The demographic status of European populations of Chinese sleeper was examined in Arlequin 3.5 (Excoffier and Lischer 2010). We assessed current demographic status with mismatch distribution, supplemented with selective neutrality tests; i.e. Tajima's *D* (Tajima 1989) and Fu's *FS* (Fu 1997) as indicators of population expansion. The genetic connectivity between different sampling sites was tested with the F_{ST} estimator (Weir and Hill 2002) in Arlequin 3.5, using default software set-ups. Analysis of molecular variance (AMOVA; Excoffier et al. 1992), with two schemes, was applied to reveal whether and how the European population of Chinese sleeper is spatially structured. In the first scheme, countries were used as the basic grouping factor for sampling sites, assuming that they may represent different and independent introduction events. In the second scheme, sampling sites were grouped according to river drainages (Table 1). As some localities were geographically close (ca. 20 km distance), we pooled them with the closest sampling site to increase the number of individuals per site and to simplify the analysis.

The relationships among the Chinese sleeper haplotypes identified during this study were analysed and graphically presented as a median-joining network with the aid of PopART 1.7 (Bandelt et al. 1999). Additionally, the phylogenetic position of these haplotypes in regards to the clades found within the native population of Chinese sleeper from China in the study by Xu et al. (2013, 2014) was reconstructed using the Maximum Likelihood approach (ML), with 1,000 bootstrap replicates, in MEGA X (Kumar et al. 2018). The HKY+G (Hasegawa-Kishino-Yano, Gamma distribution) (Hasegawa et al. 1985) was chosen, in the same software, as the best fitting substitution model. The cytochrome b sequences of *Odontobutis obscura* (AB021243), *Odontobutis potamophila* (AY722247) and *Odontobutis platycephala* (DQ010651) from GenBank (Benson et al. 2005), were used as an outgroup.

Results

The 1172 bp long portion of cytochrome b gene was sequenced from 261 Chinese sleeper individuals collected from a variety of water bodies in Belarus, Bulgaria, Hungary, Germany, Latvia, Lithuania, Poland, Russia (European part) and Ukraine (Table 1, Suppl. material 1: Table S1). We obtained 267 sequences, since six of the sequenced fish revealed double cytochrome b sequences. The latter included five individuals from Ukraine (4 ind. from site 19, 1 ind. from site 20), as well as one individual from Poland (site 10), but were excluded from our dataset. As a result, the dataset contained 255 sequences with 45 variable sites and no indels. The nucleotide diversity per site (Pi) was 0.007 (SD: 0.001), while the average number of nucleotide differences (k) was 7.9. The number of haplotypes (h) defined in our dataset equalled 22 with the haplotype diversity (Hd) of 0.685 (SD: 0.018).

A Median-Joining network revealed that the haplotypes identified in our dataset formed three major groups, with partially disjunct geographic distributions (Figs 1B, 2). Haplogroup I consisted of three haplotypes only and was found exclusively in Latvia. Haplogroup II consisted of five haplotypes. Its topology was star-like

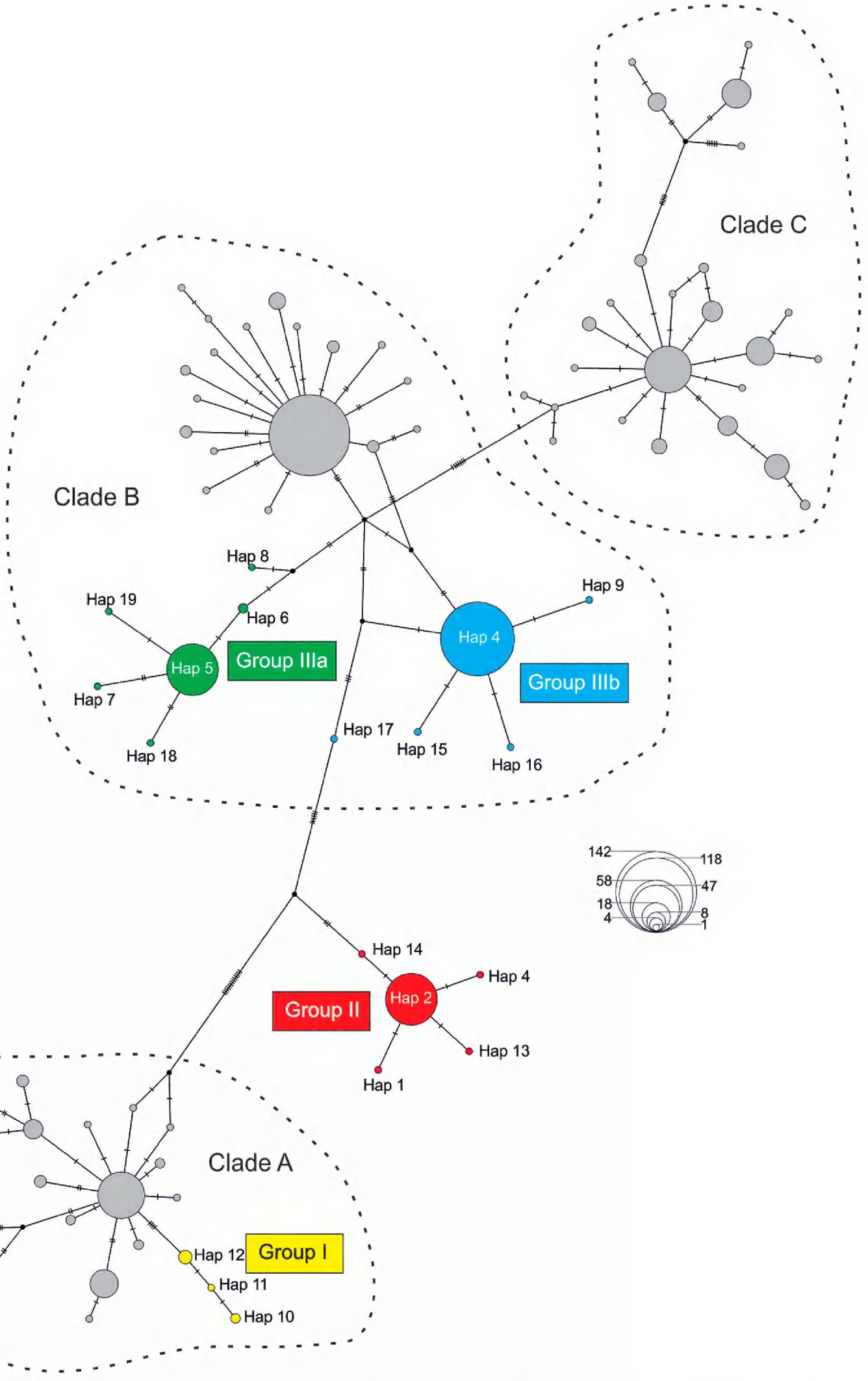

Figure 2. Median-Joining network showing phylogenetic relationships among Chinese sleeper cytochrome b haplotypes identified in our study and those (in grey) reported by Xu et al. (2014) from the native range in China. Circle size is proportional to the number of individuals with a given haplotype (see open circles with numbers).

with the central, most abundant, haplotype 2. This haplogroup was found only in the eastern part of the studied region, namely in eastern Lithuania, Latvia and Belarus as well as in northern Ukraine. Haplogroup III included as many as eleven haplotypes and had a more complex, partially star-like and partially reticulate topology. Haplotypes 4 and 5 were the most abundant. This haplogroup might be further divided into two subgroups; i.e. subgroup IIIa centred around haplotype 5, and subgroup IIIb centred around haplotype 4. Subgroup IIIa was present in southern Ukraine and, together with subgroup IIIb, at one site in Belarus. Subgroup IIIb prevailed in western Ukraine. Both subgroups were found in Poland, Bulgaria and Hungary, while only subgroup IIIa was found in the sampled sites in Germany.

Mismatch distribution analysis, accompanied by Tajima's *D* and Fu's *FS* neutrality tests (Table 2), rejected a model of recent demographic expansion for the European population of Chinese sleeper. However, the spatial expansion model could not be dismissed.

Analysis of Molecular Variance (Table 3) showed that 44.9% of the observed genetic variation may be explained by partitioning the dataset according to country of sample origin. The remaining 55.1% of variation in our dataset can be attributed to differences between the sampling sites (30.8%) and the variation within them (24.3%). Partitioning the dataset by river basin explained only 39.5% of the observed variation, while 36.1% of the variation could be accounted for by differentiation among sites.

The F_{ST} coefficient values (0.00–0.99) suggested extremely varied levels of genetic connectivity among sampling sites (Fig. 3, Suppl. material 2: Table S2). All sampled populations from Russia (site 1), Latvia (sites 2 and 3), Lithuania (site 4), two from

Table 2. Results of mismatch distribution analysis and Tajima's *D* and Fu's *FS* neutrality tests for Chinese sleeper.

	Demographic expansion	Spatial expansion
SSD*	0.5771	0.0844
SSD P -value	0.0000	0.2300
Raggedness index	0.2354	0.2354
Raggedness P-value	1.0000	0.4300
Tajima's *D*	0.2036	
Tajima's *D* P -value	0.6710	
Fu's *FS*	4.4171	
Fu's *FS* P -value	0.8810	

*SSD – the sum of squared deviations.

Table 3. Results of AMOVA for Chinese sleeper.

Source of variation	d.f.	Sum of squares	Variance components	Variance [%]	Fixation Indices
Sampling sites grouped according to countries					
Among groups	8	534.85	1.94 Va	44.87	F_{SC}: 0.56
Among populations within groups	17	225.37	1.33 Vb	30.84	F_{ST}: 0.76
Within populations	229	240.03	1.05 Vc	24.29	F_{CT}: 0.45
Total	254	1000.24	4.31		
Sampling sites grouped according to river basins					
Among groups	8	506.99	1.69 Va	39.46	F_{SC}: 0.60
Among populations within groups	17	253.23	1.55 Vb	36.10	F_{ST}: 0.76
Within populations	229	240.03	1.05 Vc	24.44	F_{CT}: 0.40
Total	254	1000.24	4.29		

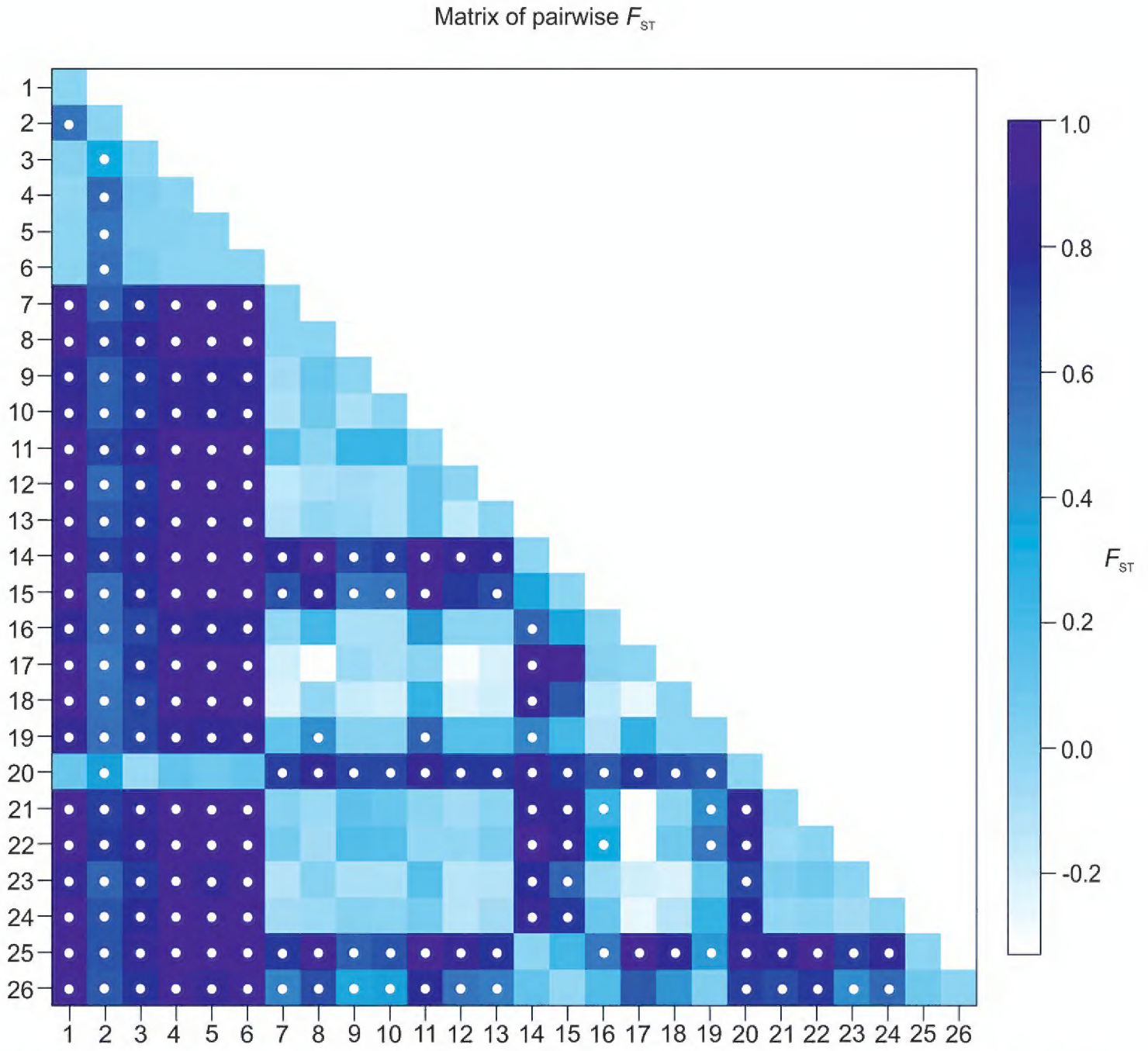

Figure 3. Population connectivity illustrated by a matrix of pairwise F_{ST} values (see also Table S2). White dots indicate F_{ST} P values significantly different from zero (P <0.05). Sites in close geographic proximity pooled for demographic analyses and assigned the same number.

Belarus (sites 5 and 6) and one from Ukraine (site 20) formed a group that was relatively isolated from all other populations, while a high level of genetic connectivity is retained within this group. A population from Germany (site 14), one from Ukraine (site 15), and two from Hungary (site 25 and 26) appeared to have no connectivity with populations from other areas. However, this finding may be related to a highly uneven sample size between some of these localities. In contrast, the remaining populations can be characterized by relatively high or moderate levels of genetic connectivity.

Phylogenetic reconstruction of relationships among the haplotypes observed in our dataset and those defined by Xu et al. (2014) in populations of Chinese sleeper from the Liaohe and Amur River basins in north-eastern China revealed that our haplogroups I and III can be attributed to Xu et al. (2014) clades A and B, respectively (Fig. 4). However, none of the haplotypes in our dataset is identical to any haplotype from Xu et al. (2014). Haplogroup II formed a separate clade, more closely related to clades B and C than to clade A (as defined by Xu et al. 2014).

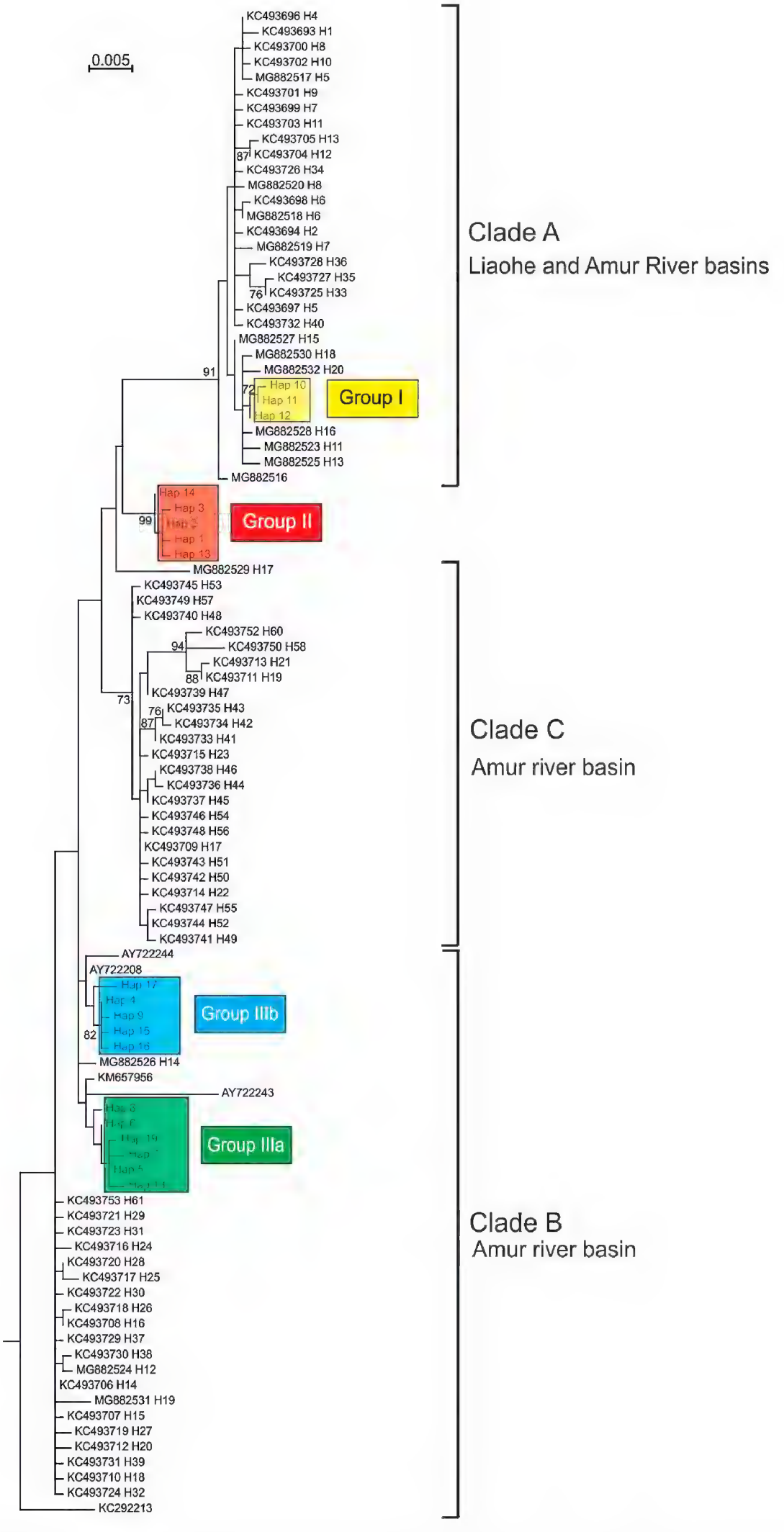

Figure 4. Maximum Likelihood tree showing the relationships of cytochrome b haplotypes identified in our study with the haplotypes from the native area in China (Xu et al. 2013, 2014). Numbers at each node are bootstrap probabilities based on 1000 replications; shown only when they are 70% or higher. Outgroup not shown.

Discussion

Our study revealed that the invasive Chinese sleeper in Europe consists of at least three distinct haplogroups that may represent independent introduction events from different parts of its native range. Haplogroup I, henceforth termed "Baltic", was found only in Latvia (Fig. 1B) in natural stagnant waters in the River Daugava drainage (Baltic Sea basin). In the native range of the Chinese sleeper, this haplogroup occurs in China in the Liaohe drainage and in the River Argun (left tributary of the River Amur) (Xu et al. 2014).

The first records of the Chinese sleeper in Latvia come from small natural ponds in the city centre of Daugavpils (Fig. 1A), that are not connected with any river system (Pupina et al. 2015). It was unknown how the species was introduced there but the population may have arisen from releases by local aquarists or fishermen (Plikšs and Aleksejevs 1998). Further investigation confirmed several sources of anthropogenic introductions in Latvia, including releases by aquarists and by anglers using them as a live bait, as well as through intentional stocking to control the density of *Carassius* sp. or as a prey for northern pike, *Esox lucius*, Linnaeus, 1758 (Pupiņš and Čeirāns, unpublished data). In addition, several Latvian websites currently offer sexually mature Chinese sleeper for purchase to stock ponds (Pupina et al. 2015; Pupiņš and Čeirāns, unpublished data). This information, combined with an absence of local haplotypes in any other population from the invasive range, strongly suggest that aquarist and angler releases of specimens translocated directly from China is possible, particularly when taking into account that China is a known exporter of ornamental pet species collected in the wild (Nijman 2010).

Another possibility is that the Chinese sleeper population in Latvia derives from a much earlier release by aquarists in Saint Petersburg in 1916 (Kuderskiy 1982), which would explain the coexistence of haplogroup I with haplogroup II that is distributed primarily in northern Europe (Fig. 1B). Unfortunately, we have no access to comparative material from that location to verify this scenario.

Haplogroup II was recorded in Lithuania (the River Neman drainage), Latvia (the River Daugava drainage), Belarus and northern Ukraine (the River Dnieper drainage) (Fig. 1B). It has not been found in any of the earlier studied Chinese populations (Xu et al. 2014). Considering the distribution of this group of haplotypes, mainly in the northern, and possibly eastern, part of its invasive range, we cannot discount the possibility that it may also be associated with the first introduction to Saint Petersburg at the beginning of the 20th century (Fig. 1A). These first individuals came from the River Zeya that is a left tributary of the River Amur and runs through the Russian Far East, but not through China (Kuderskiy 1982). Unfortunately, there is no information about the genetic structure of the Chinese sleeper from that part of its native range to indicate it as a possible source for haplotype group II.

According to the literature, after rapidly spreading in the vicinity of Saint Petersburg, most probably due to active dispersal, the species was soon found in several new locations (Kuderskiy 1982). In Lithuania, the Chinese sleeper was first recorded in

Lake Bevardis in 1985 (Virbickas 2000) (Fig. 1A). Its occurrence in coastal waters of the Gulf of Finland of the Baltic Sea (Dmitriev 1971) could allow migration in the oligohaline waters along the south-eastern Baltic coast, entering other river systems and forming secondary sources for the invasion in inland waters. However, during its initial phase of expansion in Lithuania, the Chinese sleeper was only recorded in small ponds and oxbows and not in the River Neman, so an inland expansion along the Baltic coast was probably though human releases. Only later did it spread inland via the basins of the Rivers Neman and Daugava. This scenario could explain the co-occurrence of the haplogroup II with haplogroup I in the two sampling sites in Latvia, which are located in the Daugava basin. The Chinese sleeper has been reported from the River Neman in Belarus since 2007 (Semenchenko et al. 2009) (Fig. 1A), and in 2009 it was found in the Belarusian part of the River Daugava (Lukina 2011). The spread of the Chinese sleeper in this north-eastern part of its invasive range is consistent with the distribution of haplogroup II (Fig. 1B).

Moreover, the population from the lower River Volga drainage, the only sample we obtained from Russia, also belongs to haplogroup II (Fig. 1B). The first reports of Chinese sleeper from this part of Russia come from 1970–1971, when the Chinese sleeper was accidentally inadvertently introduced with common carp, *Cyprinus carpio* Linnaeus, 1758, from the River Amur basin to the Ilev fish farm in Nizhniy Novgorod province (Fig. 1A), Volga basin, Russia (Kuderskiy 1980).

Haplogroup III was common in Ukraine and the only haplogroup found in Poland, Hungary, Bulgaria and Germany (Fig. 1B). It was also found in one locality in Belarus (the River Dnieper) (Fig. 1B). The distribution of this haplogroup strictly overlaps with the distribution of the so-called "Carpathian population" as defined by Kvach et al. (2016b), with which it is apparently identical. Thus, we retain the designation "Carpathian" for this haplogroup. It supports the proposition that the fish farm near Lviv in Ukraine, where the Chinese sleeper was first recorded in 1972 (Fedoniuk 2005) (Fig. 1B), could be the source population for many further introductions and subsequent dispersal through Central Europe (Reshetnikov 2013; Kutsokon 2017). The Chinese sleeper was introduced unintentionally to the Lviv region, Western Ukraine, during stocking of commercial fish, juvenile silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844), at the end of the 1960s (Reshetnikov 2013). The source of this introduction is unknown, but it could have been translocated directly from China or from other fish farms where Asian cyprinids were cultivated, such as those in Uzbekistan or Kazakhstan.

This scenario is not unlikely, as in 1958, the Chinese sleeper was introduced together with juvenile silver carp and grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), from Harbin (China) to the Almaty fish farm in Kazakhstan (Seleznev 1974). In 1961 it was probably transported from China to Akkurgan fish farm (River Syr Darya basin) in Uzbekistan (Borisova 1972). Haplogroup III is widely distributed in the native range of the Chinese sleeper in the northern China (Xu et al. 2014). It occurs in the River Hun (a tributary of the Liaohe) and in rivers of the River Amur system; i.e. Songhua, Argun, Nenjiang and Middle Amur, bordering Russia. It is also present in an

area close to Harbin (China), mentioned as a source from which the Chinese sleeper was collected for stocking in Kazakhstan in the 1960s. Thus, although these historical explanations for the Chinese sleeper in Uzbekistan and Kazakhstan have recently been questioned by Reshetnikov (2010), the results of our molecular studies do not exclude such a scenario for the origin of the population in Ukraine.

The later expansion of the species from Ukraine was both passive as an outcome of stocking cultivated Asian cyprinids in ponds, as well as active dispersal through aquatic networks. The Chinese sleeper was transported with silver carp to Transcarpathia, where it was first reported in the River Latorica in 1995 (Syvokhop 1998). Another route was along the River Tisza basin where it was reported in Hungary in 1997 (Harka 1998) (Fig. 1A) and in Slovakia in 1998 (Koščo et al. 1999). It expanded down the River Danube, facilitated by flooding, and was first observed in both the Bulgarian (Jurajda et al. 2006) (Fig. 1A) and Romanian section of the Danube in 2005 (Popa et al. 2006). The westernmost population of the Chinese sleeper in the Danube basin was recorded in 2009 in extensive enclosed fish ponds located in the River Naab basin, Bavaria, Germany (Reshetnikov and Schliewen 2013; Nehring and Steinhof 2015) (Fig. 1A). For decades, these ponds have been used extensively for fish production (Reshetnikov and Schliewen 2013). Reshetnikov and Schliewen (2013) assumed that the Chinese sleeper was accidentally introduced into these fish ponds with commercial fish transportation. We found only one haplotype (H5) in this population, which is the second most common and widespread in Central Europe, including Ukraine and Hungary.

In Ukraine the Carpathian population of the Chinese sleeper has spread through the Dniester basin since 1995 (Korte et al. 1999; Fedoniuk 2005; Moshu and Kiriyak 2011). In the Southern Bug (Boh) basin it has been known since 2009 from Vinnytsia (Kutsokon et al. 2014; Kutsokon 2017). In the Vistula basin, the Chinese sleeper was first reported in 1988, from the River Vyshnia in Ukraine (Movchan 1989) (Fig. 1A). In 1993 it extended its range to the Middle Vistula in Poland (Antychowicz 1994) and reached the Vistula delta in 2000 (Kostrzewa et al. 2004) (Fig. 1A). In 2006 it was found in the Shatsky Lakes in Ukraine, which connect the Vistula and Dnieper basins (Liesnik 2008) (Fig. 1A). The haplotypic composition of the Chinese sleeper population in Poland is the most similar to that in western Ukraine i.e. in the Shatsky Lakes (Fig. 1B). The route and vector of the Chinese sleeper introduction to Poland are unknown but, considering our results, unintended translocation with aquaculture stocking from Ukraine is the most probable, with commercial websites in Poland offering stocking material of carp and other species from Ukraine.

The origin of the Chinese sleeper in the River Dnieper is controversial. It was first reported from the city of Minsk in Belarus in the 1970s (Rizevski et al. 1999) (Fig. 1A). In the River Desna (left Dnieper tributary), in Russia, it was recorded in 1990 (Reshetnikov 2010) (Fig. 1A). In Ukraine (Middle Dnieper basin) it was first reported in 2001 from the vicinity of Kyiv (Sabodash et al. 2002) (Fig. 1A). It subsequently spread south, where it was found in the Dnieper tributaries (Kutsokon 2017). In the Middle Dnieper, only haplogroup IIIa was recorded, the same as in the Southern Bug (Boh) basin (Fig. 1B). This finding corroborates that Chinese sleepers from

the Dnieper and Southern Bug form one population with those from the Carpathian region, as previously hypothesized by Kvach et al. (2016b) based on the parasitological studies. We observed a decrease of genetic diversity in the Carpathian population from its presumed source (Lviv region), from where it subsequently colonised other regions (Upper Danube, Middle Vistula, Middle Dnieper, Southern Bug) (Fig. 1B).

Based on demographic analyses, such as the F_{ST} and AMOVA, we estimate the genetic connectivity between most of the Chinese sleeper populations to be high, and the molecular diversity in Central Europe showing no clear spatial structure, neither following river basins nor grouping by country. This finding suggests a rather multidirectional spread of the species in Central Europe. Interestingly, we observed a cessation of gene flow among populations from Latvia and elsewhere. This outcome suggests an independent introduction of the Chinese sleeper in Latvia and possible isolation of this population. However, more studies involving nuclear markers are needed to fully resolve this question.

In conclusion, based on the spatial distribution of mitochondrial cytochrome b diversity, we can distinguish three Chinese sleeper haplogroups in Europe, that may represent three discrete introduced populations: (1) A Baltic haplogroup that may originate from fish introduced unintentionally from Russia or from some unidentified vectors, probably releases by aquarists. To date this population was found only in the Daugava basin in Latvia. (2) East-European haplogroup, which possibly originates from an unintentional introduction to the Volga basin in Russia and with subsequent westward expansion. This group has been recorded in the Volga, Upper Dnieper and Neman drainages in Belarus, Lithuania, and Russia. (3) Carpathian haplogroup, originating from individuals inadvertently introduced with Asian cyprinid fishes to the Lviv region in Ukraine, which occurs in the basins of the Rivers Danube, Dnieper and Vistula in Belarus (eastern part), and also in Bulgaria, Germany, Hungary, Poland, and Ukraine.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Antychowicz J (1994) *Perccottus glehni* w naszych wodach. Komunikaty Rybackie 2: 21–22. [in Polish]

Bandelt H, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1): 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2005) GenBank: update. Nucleic Acids Research 33: D34–D38. https://doi.org/10.1093/nar/gkh045

Bogutskaya NG, Naseka AM (2002) *Perccottus glenii* Dybowski, 1877. Freshwater Fishes of Russia. Zoological Institute RAS. Saint Petersburg, Russia. http://www.zin.ru/Animalia/Pisces/eng/taxbase_e/species_e/perccottuss_el.htm

Bogutskaya NG, Naseka AM, Shedko SV, Vasil'eva ED, Chereshnev IA (2008) The fishes of the Amur River: updated check-list and zoogeography. Ichthyological Exploration in Freshwaters 19: 301–366.

Borisova AT (1972) Sluchaynye vselentsy v vodoemakh Uzbekistana. Voprosy Ikhtiologii 12(1): 49–53. [in Russian]

Copp GH, Bianco PG, Bogutskaya NG, Erős T, Falka I, Ferreira MT, Fox MG, Freyhof J, Gozlan RE, Grabowska J, Kováč V, Moreno-Amich R, Naseka AM, Peňáz M, Povž M, Przybylski M, Robillard M, Russell IC, Stakėnas S, Šumer S, Vila-Gispert A, Wiesner C (2005) To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21: 242–262. https://doi.org/10.1111/j.1439-0426.2005.00690.x

Cucherousset J, Olden JD (2011) Ecological impacts of non-native freshwater fishes. Fisheries 36: 215–230. https://doi.org/10.1080/03632415.2011.574578

Dmitriev MA (1971) Ostorozhno, rotan. Rybovodstvo i rybolovstvo 1: 26–27. [in Russian]

European Commission (2016) Commission Implementing Regulation (EU) 2016/1141 of 13 July 2016 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council. Official Journal of the European Union (L189): 4–8.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479–491. https://doi.org/10.1007/s00424-009-0730-7

Fedoniuk OV (2005) Holoveshka amurska (*Perccottus glenii*) v umovakh vodoym Lvivshchyny. In: Bioriznomanittia ta rol zootsenozu v prirodnykh i antropogennykh ekosystemakh: Materialy III Mizhnarodnoy naukovoyi konferentsii, Dnipropetrovsk, 4–6 zhovtnia 2005 r. Vydavnytsvo DNU (Dnipropetrovsk): 102–104. [in Ukrainian]

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147(2): 915–925

Gozlan RE, Britton JR, Cowx I, Copp GH (2010) Current knowledge on non-native freshwater fish introductions. Journal of Fish Biology 76: 751–786. https://doi.org/10.1111/j.1095-8649.2010.02566.x

Grabowska J, Błońska D, Kati S, Nagy SA, Kakareko T, Kobak J, Antal L (2019) Competitive interactions for food resources between the invasive Amur sleeper (*Perccottus glenii*) and threatened European mudminnow (*Umbra krameri*). Aquatic Conservation: Marine and Freshwater Ecosystems 29: 2231–2239. https://doi.org/10.1002/aqc.3219

Grabowska J, Grabowski M, Pietraszewski D, Gmur J (2009) Non-selective predator – the versatile diet of Amur sleeper (*Perccottus glenii* Dybowski, 1877) in Vistula River (Poland), a newly invaded ecosystem. Journal of Applied Ichthyology 25: 451–459. https://doi.org/10.1111/j.1439-0426.2009.01240.x

Grabowska J, Pietraszewski D, Przybylski M, Tarkan AS, Marszał L, Lampart-Kałużniacka M (2011) Life-history traits of Amur sleeper, *Perccottus glenii*, in the invaded Vistula River: early investment in reproduction but reduced growth rate. Hydrobiologia 661: 197–210. https://doi.org/10.1007/s10750-010-0524-0

Harka Á (1998) Magyarország faunájának új halfaja: az amurgéb (*Perccottus glehni* Dybowski, 1877). Halászat 91(1): 32–33. [in Hungarian]

Hasegawa M, Kishino H, Yano T (1985) Dating the human-ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160–174. https://doi.org/10.1007/BF02101694

Hillis DM, Moritz C, Mable BK (1996) Molecular Systematics (2nd ed.). Sinauer Associates, Sunderland, 655 pp. https://doi.org/10.2307/1447682

Hulme PE (2009) Trade, transport and trouble: Managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46: 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

Jurajda P, Vassilev M, Polačik M, Trichkova T (2006) A first record of *Perccottus glenii* (Perciformes: Odontobutidae) in the Danube River in Bulgaria. Acta Zoologica Bulgarica 58: 279–282.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Korte E, Lesnik V, Lelek A, Sondermann W (1999) Impact and overexploitation on fish community structure in the upper River Dniester (Ukraine). Folia Zoologica 48(2): 137–142.

Kostrzewa J, Grabowski M, Zięba G (2004) Nowe inwazyjne gatunki ryb w wodach Polski. Archives of Polish Fisheries 12: 21–34. [in Polish]

Koščo J, Košuth P, Hrtan E (1999) Býčkovec hlavatý (*Perccottus glehni*), ďalší nový prvok ichtyofauny Slovenska. Poľovníctvo a Rybárstvo 51(6): 1–33. [in Slovakian]

Koščo J, Lusk S, Halačka K, Lusková V (2003) The expansion and occurrence of the Amur sleeper (*Perccottus glenii*) in eastern Slovakia. Folia Zoologica 52: 329–336.

Koščo J, Manko P, Miklisová D, Košuthová L (2008) Feeding ecology of invasive *Perccottus glenii* (Perciformes, Odontobutidae) in Slovakia. Czech Journal of Animal Science 53: 479–486. https://doi.org/10.17221/340-CJAS

Kottelat M, Freyhof J (2007) Handbook of European Freshwater Fishes. Publications Kottelat, Cornol and Freyhof, Berlin, 646 pp.

Kuderskiy LA (1980) Rotan v prudakh Gorkovskoy oblasti. In: Rybokhoziaystvennoye izucheniye vnutrennikh vodoyemov. Sbornik Nauchnykh Trudov GosNIORKh 25: 28–33 [in Russian]

Kuderskiy LA (1982) Rotan v prudakh Leningradskoy oblasti. Sbornik Nauchnykh Trudov GosNIORKh 191: 70–75. [in Russian]

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Kutsokon I (2017) The Chinese sleeper (*Perccottus glenii* Dybowski, 1877) in Ukraine: new data on distribution. Journal of Applied Ichthyology 33(6): 1100–1107. https://doi.org/10.1111/jai.13454

Kutsokon Y, Tsyba A, Kvach Y (2014) The occurrence of the Chinese sleeper *Perccottus glenii* Dybowski, 1877 in the Southern Bug River basin, Ukraine. BioInvasions Records 3(1): 45–48. https://doi.org/10.3391/bir.2014.3.1.08

Kvach Y, Dykyy I, Janko K (2016a) First record of the Chinese sleeper, *Perccottus glenii* Dybowski, 1877 (Actinopterygii: Odontobutidae) in the Dnieper Estuary, southern Ukraine (Black Sea drainage). BioInvasions Records 5(4): 285–290. https://doi.org/10.3391/bir.2016.5.4.14

Kvach Y, Kutsokon Y, Stepien CA, Markovych M (2016b) Role of the invasive Chinese sleeper *Perccottus glenii* Dybowski 1877 (Actinopterygii: Odontobutidae) in the distribution of fish parasites in Europe: new data and a review. Biologia 71(8): 941–951. https://doi.org/10.1515/biolog-2016-0112

Leunda PM (2010) Impacts of non-native fishes on Iberian freshwater ichthyofauna: current knowledge and gaps. Aquatic Invasions 5: 239–262. https://doi.org/10.3391/ai.2010.5.3.03

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Liesnik VV (2008) Rybni uhrupovannia malykh vodoym Shatskoho natsionalnoho pryrodnoho parku. In: Stan i bioriznomanittia ekosystem Shatskoho natsionalnoho pryrodnoho parku: Materialy naukovoyi konferensii, Shatsk, 11–14 veresnia 2008 r. Spolom (Lviv): 85–86. [in Ukrainian]

Luca M, Ureche D, Nicuță D, Ghiorghiță G, Druică RC, Gorgan LD (2014) The genetic variability of the invasive *Perccottus glenii* from Siret River, using the cytochrome b gene. Annals of the Romanian Society for Cell Biology 19(1): 11–20.

Lukina II (2011) Distribution of the Amur sleeper (*Perccottus glenii* Dybowski, 1877) in Belarus. Russian Journal of Biological Invasions 2(2–3): 209–212. https://doi.org/10.1134/S2075111711030088

Miller PJ, Vasil'eva ED (2003) *Perccottus glenii* Dybowski 1877. In: Miller PJ (Ed.) The Freshwater Fishes of Europe (Vol. 8/1). Aula-Verlag, Weibelsheim, 135–156.

Mori T (1936) Studies on the Geographical Distribution of Freshwater Fishes in Eastern Asia. Toppan Print, Tokio, 88 pp.

Moshu A, Kiriak D (2011) Rasprostranenie rotana-goloveshki – *Perccottus glenii* Dybowski, 1877 (Perciformes: Odontobutidae) v vodoemakh Prut-Dnestrovskogo mezhdurechya. Academician Leo Berg – 135 years: Collection of Scientific Articles. EcoTyras, Bendery, 415–420. [in Russian]

Movchan YV (1989) Pervaya nakhodka goloveshki (rotana) – *Percottus glehni* Dybowski (Pisces, Eleotridae) v vodoyemakh Ukrainy. Vestnik Zoologii 5: 1–87. [in Russian]

Nabatov AA (1914) Komnatnyi Presnovodnyi Akvarium. Akvarium, Petrograd, 532 pp. [in Russian]

Nehring S, Steinhof J (2015) First records of the invasive Amur sleeper, *Perccottus glenii* Dybowski, 1877 in German freshwaters: a need for realization of effective management measures to stop the invasion. BioInvasions Records 4(3): 223–232. https://doi.org/10.3391/bir.2015.4.3.12

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York, 522 pp. https://doi.org/10.7312/nei-92038

Nijman V (2010) An overview of international wildlife trade from Southeast Asia. Biodiversity Conservation 19: 1101–1114. https://doi.org/10.1007/s10531-009-9758-4

Plikšs M, Aleksejevs E (1998) Zivis. Gandrs, Riga, 304 pp. [in Latvian]

Popa LO, Popa OP, Pisică EI, Iftime A, Matacă S, Diaconu F, Murariu D (2006) The first record of *Perccottus glenii* Dybowski, 1877 (Pisces: Odontobutidae) and *Ameiurus melas* Rafinesque, 1820 (Pisces: Ictaluridae) from the Romanian sector of the Danube. Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa" 49: 323–329.

Pupina A, Pupins M, Skute A, Pupina Ag, Karklins A (2015) The distribution of the invasive fish Amur sleeper, rotan *Perccottus glenii* Dybowski, 1877 (Osteichthyes, Odontobutidae), in Latvia. Acta Biologica Universitatis Daugavpiliensis 15(2): 329–341.

Pupins M, Pupina A (2012) Invasive fish *Perccottus glenii* in biotopes of *Bombina bombina* in Latvia on the north edge of the fire-bellied toad's distribution. Acta Biologica Universitatis Daugavpiliensis (Supplement 3): 82–90

Pupins M, Pupina A (2018) Reciprocal predation between preserved and invasive species: adult *Bombina bombina* predate young white baits of alien fish *Perccottus glenii*. Acta Biologica Universitatis Daugavpiliensis 18(2): 217–223.

Rakauskas V, Masiulytė R, Pikūnienė A (2016a) Predator-prey interactions between a recent invader, the Chinese sleeper (*Perccottus glenii*) and the European pond turtle (*Emys orbicularis*): a case study from Lithuania. Acta Herpetologica 11: 101–109. https://doi.org/10.13128/Acta_Herpetol-18261

Rakauskas V, Stakėnas S, Virbickas T, Bukelskis E (2016b) Non-indigenous fish in the northern branch of the central European invasion corridor. Reviews in Fish Biology and Fisheries 26(3): 491–508. https://doi.org/10.1007/s11160-016-9438-x

Ratnasingham S, Hebert PD (2007) BOLD: The barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Resources 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rechulicz J, Płaska W, Nawrot D (2015) Occurrence, dispersion and habitat preferences of Amur sleeper (*Perccottus glenii*) in oxbow lakes of a large river and its tributary. Aquatic Ecology 49: 389–399. https://doi.org/10.1007/s10452-015-9532-5

Reshetnikov AN (2004) The fish *Perccottus glenii*: history of introduction to western regions of Eurasia. Hydrobiologia 522: 349–350. https://doi.org/10.1023/B:HYDR.0000030060.29433.34

Reshetnikov AN (2010) The current range of Amur sleeper *Perccottus glenii* Dybowski, 1877 (Odontobutidae, Pisces) in Eurasia. Russian Journal of Biological Invasions 1(2): 119–126. https://doi.org/10.1134/S2075111710020116

Reshetnikov AN (2013) Spatio-temporal dynamics of the expansion of rotan *Perccottus glenii* from West-Ukrainian center of distribution and consequences for European freshwater ecosystems. Aquatic Invasions 8(2): 193–206. https://doi.org/10.3391/ai.2013.8.2.07

Reshetnikov AN, Ficetola GF (2011) Potential range of the invasive fish rotan (*Perccottus glenii*) in the Holarctic. Biological Invasions 13: 2967–2980. https://doi.org/10.1007/s10530-011-9982-1

Reshetnikov AN, Schliewen UK (2013) First record of the invasive alien fish rotan *Perccottus glenii* Dybowski, 1877 (Odontobutidae) in the Upper Danube drainage (Bavaria, Germany). Journal of Applied Ichthyology 29: 1367–1369. https://doi.org/10.1111/jai.12256

Rizevski VK, Pliuta MV, Ermolaev VV (1999) Morfologicheskaia kharakteristika rotana-goloveshki (*Perccottus glenii* Dybowski) iz vodoemov vodnoy sistemy Minska. Vestsi Natsiyanalnay akademii navuk Belarusi (Seria Biological Sciences) 3: 119–121. [in Russian]

Sabodash VM, Tkachenko VA, Tsyba AA (2002) Finding of a population of rotan *Perccottus glenii* (Pisces, Odontobutidae) in water bodies of the Kiev Oblast. Vestnik Zoologii 36(2): 1–90. [in Russian]

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Seleznev VV (1974) Malotsennye i sornye vidy ryb kitayskogo kompleksa v Kapchagayskom vodokhranilishche. In: Rybnye resursy vodoemov Kazakhstana i ikh ispolzovanie, Issue 8. Kainar (Alma-Ata): 143–148. [in Russian]

Semenchenko VP, Rizevsky VK, Mastitsky SE, Vezhnovets VV, Pluta MV, Razlutsky VI, Laenko T (2009) Checklist of aquatic alien species established in large river basins of Belarus. Aquatic Invasions 4(2): 337–347. https://doi.org/10.3391/ai.2009.4.2.5

Syvokhop YM (1998) Pershi znakhidky rotana (*Perccottus glenii* Dybowski) na Zakarpatti. Tezy Studentskoyi Konferentsii, Seria Biologia, Patent No 3. Vydavnytsvo UDU, Uzhgorod, 44–45. [in Ukrainian]

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123(3): 585–595.

Weir BS, Hill WG (2002) Estimating F-statistics. Annual review of genetics 36: 721–750. https://doi.org/10.1146/annurev.genet.36.050802.093940

Virbickas J (2000) Lietuvos žuvys. Ekologijos institutas, Vilnius, 192 pp. [in Lithuanian]
Vitule JRS, Freire CA, Simberloff D (2009) Introduction of nonnative freshwater fish can certainly be bad. Fish and Fisheries 10: 98–108. https://doi.org/10.1111/j.1467-2979.2008.00312.x
Xu W, Hou GY, Li CY, Kong XF, Zheng XH, Li JT, Sun XW (2013) Complete mitochondrial genome of Chinese sleeper, *Perccottus glenii*. Mitochondrial DNA 24(4): 339–341. https://doi.org/10.3109/19401736.2012.760081
Xu W, Yin W, Chen A, Li J, Lei G, Fu C (2014) Phylogeographical analysis of a cold-temperate freshwater fish, the Chinese sleeper (*Perccottus glenii*) in the Amur and Liaohe river basins of Northeast Asia. Zoological Science 31(10): 671–679. https://doi.org/10.2108/zs130046

Supplementary material 1

Table S1. Table presents Chinese sleeper haplotypes frequency (belonging to three distinguished groups) found in studies sites.

Authors: Tomasz Rewicz, Michał Grabowski, Joanna Grabowska

Data type: distribution

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Link: https://doi.org/10.3897/neobiota.57.48958.suppl1

Supplementary material 2

Table S2. Values of F_{ST} population pairwise.

Authors: Tomasz Rewicz

Data type: statistical data

Explanation note: Statistically significant values are shown in bold ($P \leq 0.05$).

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